



PATENT

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

APPLICANT(S): John Reynolds
APPLICATION NO.: 10/009,389
FILING DATE: 20 July 2002
TITLE: Transformation of *Allium sp.* with *Agrobacterium* Using
Embryogenic Callus Cultures
EXAMINER: Georgia L. Helmer
GROUP ART UNIT: 1638
ATTY. DKT. NO.: 34703/0042

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37 C.F.R. § 1.132 DECLARATION TRAVERSING REJECTION

I, John F. Reynolds, hereby declare:

that I am the inventor named of the subject matter claimed in the above-identified patent application:

that I have reviewed the Office Action dated November 3, 2005, rejecting the patent application;

that I have reviewed the prior art references and arguments cited therein;

that I have reviewed records relating to the development of the subject matter claimed in the application, namely, results of experiments relating to the transformation of *Allium sp.* with *Agrobacterium* using embryogenic callus cultures;

that the attached photocopy provides the results of a PCR experiment demonstrating the development of transformed *Allium cepa* lines in accordance with methods of the specification;

that the PCR experiment involved four transformed *Allium cepa* lines resistant to the herbicide glyphosate (lanes 2-5), one non-transformed *Allium cepa* line (lane 6), and a construct

transformed *Allium cepa* line (lane 6), and a construct having the heterologous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene (lane 7), with lanes 1 and 8 being molecular weight markers;

that the transformed *Allium cepa* line was developed over the course of the last several years using techniques and starting materials set forth in the specification, namely, by a) culturing immature embryos from *Allium cepa* on an initiation medium for a period of from 2 to 6 months until an embryogenic callus formed, b) transferring the embryonic callus to a coculture medium and contacting the embryogenic callus with a suspension of *Agrobacterium tumefaciens* containing a DNA of interest from a heterologous gene, and c) obtaining a transformed *Allium cepa* embryogenic callus under selective conditions;

that the construct used in development of the transformed *Allium cepa* lines is the one described in Example 2 of the patent application, namely, pMON45312, that the DNA of interest was an EPSPS gene, and that the selective conditions used included growth of the transformed *Allium cepa* embryogenic callus on glyphosate;

that the PCR primers used in the PCR experiment were i) from the EPSPS gene, as a first set of PCR primers, and ii) from an onion seedling cDNA clone from GenBank number BE205672 submitted by J.A. McCallum et al. Theor. Appl. Genet. 103 (67), 979-991 (2001) used as a marker for onion genetic analysis, as a second set of PCR primers;

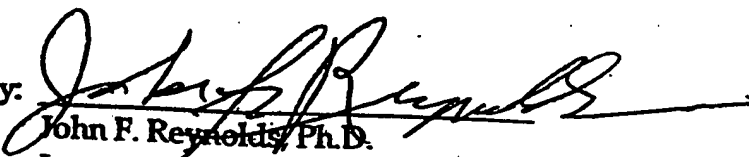
that the attached photocopy shows that the glyphosate resistant *Allium cepa* plants (lanes 2-5) each have been transformed to contain DNA of the heterologous EPSPS gene (lane 7), as evidenced by the common band generated by the first set of PCR primers;

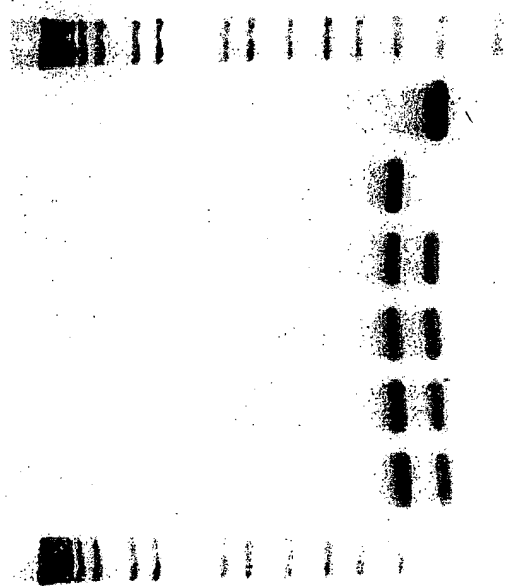
that the attached photocopy shows that the control *Allium cepa* plant (lane 6) does not contain DNA of the heterologous EPSPS gene, but only DNA of the onion cDNA clone, as evidenced by the common band between all onion plants (lanes 2-6) generated by the second set of PCR primers; and

that I, being warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon, declares that all statements made of the my own knowledge are true and that all statements made on information and belief are believed to be true.

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Dated: 4.1.2005

By: 
John F. Reynolds, Ph.D.
Inventor



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